

CLAIMS

1. A method of identifying a compound which modulates the activity to different extents of (a) a host yeast cell protein kinase or kinases and (b) an
5 protein kinase derivable from a source other than the said host yeast cell that is equivalent to the said host yeast cell protein kinase or kinases, wherein a compound is exposed to

1) a first host yeast cell wherein the yeast cell is capable of expressing the said host yeast cell protein kinase or kinases and is not capable of expressing the
10 said equivalent protein kinase
and

2) a second host yeast cell wherein the yeast cell is (a) not capable of expressing the said host yeast cell protein kinase or kinases and (b) is capable of expressing the said equivalent protein kinase derivable from a source other
15 than the host yeast cell

and the effect of the compound on the phenotype of the said yeast cells is measured, wherein either

(1) the host yeast cell is a pathogenic yeast and the source other than the host yeast cell is any source other than the host yeast cell
20 or

(2) the host yeast cell is any yeast and the source other than the host yeast cell is not a mammal.

2. A method of identifying a compound which modulates the activity to
25 different extents of (a) a protein kinase derivable from a first source and (b) a protein kinase derivable from a second source, both said protein kinases being equivalent to the same host yeast cell protein kinase or kinases, wherein a compound is exposed to

- 1) a first host yeast cell wherein the yeast cell is (a) not capable of expressing the said yeast cell protein kinase or kinases and (b) is capable of expressing the said equivalent protein kinase derivable from the first source and
- 5 2) a second host yeast cell wherein the yeast cell is (a) not capable of expressing the said yeast cell protein kinase or kinases and (b) is capable of expressing the said equivalent protein kinase derivable from the second source and the effect of the compound on the phenotype of the said yeast cells is measured.
- 10 3. A method according to claim 1 or 2 wherein the host yeast cell other than the host yeast cell that is a pathogenic yeast is from any one of the genera *Saccharomyces*, including *Saccharomyces cerevisiae*, *Candida*, including *Candida albicans*, *Pichia*, *Kluyveromyces*, *Torulopsis*, *Hansenula*,
15 *Schizosaccharomyces*, *Citeromyces*, *Pachysolen*, *Debaromyces*, *Metschnikowia*, *Rhodsporidium*, *Leucosporidium*, *Botryoascus*, *Sporidiobolus*, *Endomycopsis*, *Aspergillus*, including *Aspergillus fumigatus*, *Cryptococcus*, including *Cryptococcus neoformans*, and *Histoplasma*, including *Histoplasma capsulatum*.
- 20 4. A method according to any one of claims 1 to 3 wherein a yeast host cell which is not capable of expressing the said host yeast cell protein kinase or kinases is substantially not capable of growing unless the said yeast host cell is capable of expressing the said protein kinase derivable from a source other than
25 the said host yeast cell that is equivalent to the said host yeast cell protein kinase or kinases.
5. A method according to any one of claims 1 to 4 wherein at least one protein kinase from a source other than the host yeast cell is a human protein kinase.

6. A method according to any one of claims 1 to 5 wherein the said host yeast cell protein kinase or kinases is Pkh1 and/or Pkh2, wherein Pkh1 is the polypeptide encoded by open reading frame YDR490c of *S. cerevisiae* or
5 equivalent open reading frame in yeast other than *S. cerevisiae* and Pkh2 is the polypeptide encoded by open reading frame YOL100w of *S. cerevisiae* or equivalent open reading frame in yeast other than *S. cerevisiae*.
7. A method according to any one of claims 1 to 6 wherein the said protein
10 kinase equivalent to the said host yeast cell protein kinase or kinases is PDK1.
8. A method according to any one of claims 1 to 5 wherein the said host yeast cell protein kinase or kinases is Ypk1 and/or Yrk2.
- 15 9. A method according to any one of claims 1 to 5 and 8 wherein the said protein kinase equivalent to the said host yeast cell protein kinase or kinases is serum and glucocorticoid induced protein kinase (SGK) or protein kinase B (PKB).
- 20 10. The method of any one of claims 2 to 9 wherein the first source is a human and the second source is a pathogenic yeast from any one of the genera *Candida* spp, *Blastomyces* spp, for example *B. dermatitidis*, *Coccidioides* spp, for example *C. immitis*, *Histoplasma* spp, for example *H. capsulatum*, *Sporothrix* spp, for example *S. schenckii*, *Aspergillus* spp, for example *A. fumigatus*, *A. flavus*, *A. niger*, *Phialophora compacta* (*Fonsecaea compacta*),
25 *P. pedrosoi* (*F. pedrosi*), *P. verrucosa*, *Cladosporium carrionii*, *Rhinocladiella aquaspersa*, *Cryptococcus* spp, for example *C. neoformans*, *Cephalosporium* spp, *Fusarium* spp, *Histoplasma* spp, for example *H. capsulatum*, *Pneumocystis carinii*, *Rhizopus* spp, *Rhizomucor* spp, *Madurella* spp, for

example *M. mycetomatis*, *M. grisea*, *Pseudallescheria boydii*, *Paracoccidioides* spp, for example *P. brasiliensis*, *Prototheca* spp, for example *P. wickerhamii*, *Epidermophyton* spp, *Microsporum* spp, *Trichophyton* spp, *Malassezia* spp, for example *M. furfur* (*Pityrosporum orbiculare*)

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11. A method of identifying a compound that modulates (inhibits) the activity of PDK1 derivable from a first source, wherein a compound is exposed to

1) a first host yeast cell wherein the yeast cell is (a) not capable of expressing a yeast polypeptide that is a functional equivalent of human PDK1 (Pkh1 and
10 Pkh2) and (b) is capable of expressing PDK1 derivable from the said first source

and optionally

2) a second host yeast cell wherein the yeast cell is capable of expressing a yeast polypeptide that is a functional equivalent of human PDK1 (Pkh1 and/or
15 Pkh2)

and the effect of the compound on the viability of the said yeast cell or cells is measured, and a compound that affects the viability of the first said yeast cell, or optionally that affects the viability of the first said yeast cell and the said second yeast cell differently, is identified.

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12. A method of identifying a compound that modulates (inhibits) the activity of a functional equivalent of Ypk1 and/or Ykr2 derivable from a first source, wherein a compound is exposed to

1) a first host yeast cell wherein the yeast cell is (a) not capable of expressing a yeast polypeptide that is a functional equivalent of Ypk1 and/or Yrk2 and (b) is
25 capable of expressing a functional equivalent of Ypk1 and/or Ykr2 (for example SGK) derivable from the said first source

and optionally

2) a second host yeast cell wherein the yeast cell is capable of expressing a yeast polypeptide (for example, an endogenous polypeptide) that is a functional equivalent of Ypk1 and/or Yrk2

and the effect of the compound on the viability of the said yeast cell or cells is measured, and a compound that affects the viability of the first said yeast cell, or optionally that affects the viability of the first said yeast cell and the said second yeast cell differently, is identified.

13. A yeast cell that is not capable of expressing Pkh1 and Pkh2 or any functional equivalent thereof.

14. A yeast cell that is not capable of expressing endogenous Pkh1 and/or Pkh2.

15. A yeast cell according to claim 14 that is capable of expressing a functional equivalent of Pkh1 and/or Pkh2 that is not endogenous Pkh1 or Pkh2.

16. The yeast cell of claim 15 wherein the said functional equivalent is human PDK1 or a variant, fusion or derivative thereof.

17. A yeast cell according to any one of claims 13 to 15 wherein the open reading frame encoding Pkh1 or Pkh2 is disrupted by insertion of a selectable marker.

18. A yeast cell wherein one or more genes encoding a functional equivalent of human PDK1 is mutated such that the yeast cell is not capable of expressing the said functional equivalent of human PDK1.

19. A yeast cell according to claim 18 wherein each such gene encoding a functional equivalent of human PDK1 is mutated such that the yeast cell is not capable of expressing a functional equivalent of human PDK1.

5 20. A method of identifying a compound that modulates (inhibits) the activity of PDK1 wherein a yeast cell according to any one of claims 13 to 19 is used.

10 21. Use of a yeast cell according to any one of claims 13 to 19 in a method of identifying a compound that modulates (inhibits) the activity of PDK1.

22. The method of claim 20 or use of claim 21 wherein the PDK1 is mammalian PDK1.

15 23. The method of claim 20 or use of claim 21 wherein the PDK1 is a yeast PDK1, for example *Candida* PDK1.

20 24. A protein kinase derivable from yeast capable of phosphorylating a polypeptide comprising the consensus sequence Arg-Xaa-Arg-Xaa-Xaa-(Ser/Thr)-Hyd.

25. A protein kinase derivable from yeast capable of being phosphorylated by Pkh1 or Pkh2 or PDK1.

25 26. A protein kinase according to claim 24 or 25 wherein the said protein kinase is Ypk1 from *S. cerevisiae* or equivalent open reading frame in yeast other than *S. cerevisiae*, for example *Candida* spp or Ykr2 from *S. cerevisiae* or equivalent open reading frame in yeast other than *S. cerevisiae*, for example *Candida* spp.

27. A variant, derivative, fragment or fusion or a fusion of a variant, derivative or fragment of a protein kinase as defined in claim 26 that is capable of being phosphorylated by Pkh1 or Pkh2 or mammalian, preferably human, PDK1 and/or capable of phosphorylating a polypeptide comprising the consensus sequence Arg-Xaa-Arg-Xaa-Xaa-Ser/Thr-Hyd.

28. A yeast, for example *S. cerevisiae* or *Candida*, cell wherein one or more endogenous genes encoding a functional equivalent of human SGK is mutated such that the yeast cell is not capable of expressing the said functional equivalent of human SGK.

29. A yeast cell according to claim 28 wherein the said gene is Ypk1 or Yrk2.

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30. A yeast cell according to claim 27 or 29 wherein each such endogenous gene encoding a functional equivalent of human SGK or Ypk1 or Yrk2 is mutated such that the yeast cell is not capable of expressing an endogenous functional equivalent of, for example, human SGK or Ypk1 or Yrk2.

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32. A method of identifying a compound which blocks the activation of a polypeptide that is a functional equivalent of Ypk1 and/or Ykr2 and is not SGK, PKB α or p70S6 kinase by an interacting polypeptide, for example Pkh1, Pkh2 or PDK1, the method comprising determining whether a compound enhances or disrupts the interaction between (a) a polypeptide that is a functional equivalent of Ypk1 and/or Ykr2 that is not SGK, PKB α or p70S6 kinase or a suitable fragment, variant, derivative or fusion thereof or a suitable fusion of a fragment, variant or derivative and (b) the interacting polypeptide,

or a suitable variant, derivative, fragment or fusion thereof or a suitable fusion of a variant, derivative or fragment, or determining whether the compound substantially blocks activation of the said polypeptide that is a functional equivalent of Ypk1 and/or Ykr2 or a suitable variant, fragment, derivative or fusion thereof, or a fusion of a said fragment, derivative or fusion by the interacting polypeptide, or a suitable variant, derivative, fragment or fusion thereof.

33. The use of Pkh1 or Pkh2 or a suitable variant, fragment, derivative or fusion thereof, or a fusion of a said fragment, derivative or fusion thereof that is not PDK1 to phosphorylate and/or activate a polypeptide that is Ypk1 and/or Ykr2 or SGK or PKB α or a functional equivalent thereof or suitable variant, fragment, derivative or fusion thereof, or a fusion of a said fragment, derivative or fusion.

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34. The use of PDK1 or a suitable variant, fragment, derivative or fusion thereof, or a fusion of a said fragment, derivative or fusion thereof to phosphorylate and/or activate a polypeptide that is Ypk1 and/or Ykr2 or SGK or a functional equivalent thereof or suitable variant, fragment, derivative or fusion thereof, or a fusion of a said fragment, derivative or fusion that is not PKB α or p70S6 kinase.

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35. A kit of parts comprising means useful for carrying out the method as defined in any one of Claims 1 to 10.

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36. A kit of parts according to claim 35 comprising a first host yeast cell wherein the yeast cell is (a) not capable of expressing the said yeast cell protein kinase or kinases and (b) is capable of expressing the said equivalent protein kinase derivable from a first source

and

- 2) a second host yeast cell wherein the yeast cell is (a) not capable of expressing the said yeast cell protein kinase or kinases and (b) is capable of expressing the said equivalent protein kinase derivable from an source other
5 than the first source.

37. Any novel protein kinase as herein disclosed.

38. A compound identifiable by the method of any one of claims 1 to 12, 20,
10 22, 23 or 32.

39. A compound according to claim 38 that is capable of inhibiting mammalian PDK1 or SGK.

15 40. A compound according to claim 38 that is capable of inhibiting a fungal functional equivalent of PDK1 (which may be Pkh1 or Pkh2) or SGK (which may be Ypk1 or Yrk2).

20 41. A compound according to any one of claims 38 to 40 for use in medicine.

42. Use of a compound according to claim 40 in the manufacture of a medicament for the treatment of a fungal, for example a *Candida* infection, for example thrush.

25 43. Use of a compound according to claim 39 in the manufacture of a medicament for the treatment of cancer.

44. A substantially pure polypeptide encoded by open reading frame YDR490c of *S. cerevisiae* or equivalent open reading frame in yeast other than

S. cerevisiae or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant or fragment or derivative

or

a substantially pure polypeptide encoded by open reading frame YOL100w of
5 *S. cerevisiae* or equivalent open reading frame in yeast other than *S. cerevisiae*.

or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant or fragment or derivative wherein the polypeptide does not comprise the amino acid sequence of human PDK1 or *Drosophila* PDK1 (DSTPK61).

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45. A recombinant polynucleotide suitable for expressing a polypeptide as defined in claim 44.

15 46. A host cell comprising a recombinant polynucleotide as defined in claim 45.

20 47. A method of making a polypeptide, or a variant, fragment, derivative or fusion thereof or fusion of a said variant or fragment or derivative the method comprising culturing a host cell as defined in Claim 46 which expresses said polypeptide, or a variant, fragment, derivative or fusion thereof or fusion of a said variant or fragment or derivative and isolating said polypeptide or a variant, fragment, derivative or fusion thereof or fusion of a said variant, or fragment or derivative.

25 48. A polypeptide, or a variant, fragment, derivative or fusion thereof or fusion of a said variant or fragment or derivative obtainable by the method of Claim 47.

49. An antibody reactive towards a polypeptide as defined in claim 44 or 48.